Original Article

Absolute Lymphocyte Counts, Cd3$^+$ And Cd4$^+$ T- Lymphocyte Subsets in Adult Patients with Sickle Cell Anaemia in Zaria, Nigeria

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Abstract

Background: Impaired immunological function such as a reduction in the T cell component has been reported in patients with Sickle cell anaemia (SCA), leading to loss of both humoral and cell-mediated immunity. These patients become susceptible to infection leading to increased morbidity and mortality. **Aims**: To determine and review the level of Absolute lymphocyte counts (ALC) as well as CD3$^+$ and CD4$^+$ T-lymphocyte subsets in adult patients with SCA in our locality. **Materials and Method:** A comparative cross-sectional study of 60 participants consecutively enrolled as follows: 30 constituting the Study group (HbSS) in steady state (asymptomatic for at least 4 weeks) and 30 unmatched Controls (HbAA). Both HbSS patients and controls were HIV negative. Both groups also had automated complete blood counts and flowcytometry (BD FACS Count) for CD3+ and CD4+ T lymphocytes conducted. **Results:** The mean ALC (4.73 cells x 10^9/l ±1.5) of the Study group was significantly higher than that of the Control (1.98 cells x 10^9/l ±0.9; p =<0.0001). However, despite the significantly high ALC in the Study group, the mean CD3+ and CD4+ T lymphocyte subsets were reduced (2438µl ±843 and 1364µl ±521.3 respectively) compared to that of the Control (2673 µl ±790, p =0.27 and 1697 µl ±569, p =0.022 respectively). In contrast CD3+ and CD4+ cells were significantly correlated with ALC (r 0.792 p= 0.0001 and 0.641 p= 0.0001 respectively). **Conclusion:** Patients with Sickle Cell Anaemia in the study, show a reduced CD3+ and CD4+ T cell count despite a high peripheral absolute lymphocyte count which may be responsible for increased susceptibility to infections in SCA. In light of this peculiar immune profile demonstrated in the study, it is therefore recommended to consider the functionality of CD4+ T lymphocytes, the potential of other peripheral blood mononuclear cells as predictive of infection and splenic status in patients with Sickle Cell anaemia for further studies.

Keywords: Sickle Cell Anaemia, Lymphocytes: Absolute Count, CD3+ & CD4+ T-cells

INTRODUCTION

Globally the prevalence of Sickle cell disease (SCD) is estimated at around 20-25 million individuals worldwide, out of which 12-15 million are in sub-Saharan Africa.[1, 2] In Nigeria, more than 150,000 children are born with the disease annually and 4 million people are afflicted.[2-4] With the inheritance of the homozygous sickle β-globin gene (Hb SS), Sickle cell anaemia (SCA) is the most common monogenetic disease and affects approximately 2% of Nigerians.[5], Although haemoglobin S (HbS) polymerization and vaso-occlusion are central to the pathogenesis of SCA, overlapping pathways implicated in SCA-related endothelial dysfunction include haemolysis, defects

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in nitric oxide metabolism, ischaemia-reperfusion injury, oxidative inflammatory and coagulation mediators.[4] Several defects in immunological function have been described in SCA, and a decrease in T cell counts in 50% of patients with SCA compared to normal controls have been reported.[6, 7] As such, patients with SCA have an increased risk of developing overwhelming bacterial infection.[8, 9] Lymphocytes are of fundamental importance in immune system, as they determine the specificity of the immune response to infectious microorganisms and other foreign substances.[8, 9] They are a heterogeneous population of cells consisting of three major types: T lymphocytes (CD3+), B lymphocytes (CD19+) and Natural Killer lymphocytes (CD56+/CD3–); CD3+ cells are all T lymphocytes, which includes both CD4+ (Helper T cells) and CD8+ (Cytotoxic T cells) lymphocyte and these are the major cells involved in conferring cell mediated immune response especially against infections.[8, 9.10, 11] A lymphocyte count is usually part of a peripheral complete blood cell count which is expressed as the percentage of lymphocytes to the total number of white blood cells counted and absolute lymphocyte count deduced. [10, 11]

Pertinently, studies of peripheral absolute lymphocyte counts, and T-lymphocyte subsets have been conducted to establish reference values of CD3+ and CD4+ lymphocyte subsets, as well as CD4+/CD8+ lymphocyte ratios in healthy adults. [12, 13, 14] This study thus proposes to evaluate the absolute peripheral leukocyte counts, as well as CD3+ and CD4+ T cell subsets in adult patients with SCA for reference purposes and immunological profiling, with a view to substantiate the level of T cells in patients with SCA in our locality: Zaria. This is necessary for disease surveillance, provision of a rationale towards infection prophylaxis and prompt therapeutic intervention.

MATERIALS AND METHODS

Study Area, Study Design and Participant Recruitment

This was a comparative cross-sectional study carried out at the Haematology Day clinic and Blood donation unit of Ahmadu Bello University Teaching Hospital (ABUTH) Zaria, Nigeria over a period of three months: January to March, in 2017.

The participants within the age range 18-50 years old were enrolled consecutively; 30 adult patients with SCA (HbSS) in "steady state" (steady haematocrit and haemoglobin values over 2 to 3 months and a state of well-being, without any symptoms or signs of HIV or other overt infection, pain, or any other acute episode suggestive of crisis, as established by a careful history and a complete physical examination) as Study [15, 16] and 30 prospective apparently healthy blood donors with normal (HbAA) as Control with comparatively similar age (± 3yrs) and sex to the Study. Socio-demographic characteristics were obtained using a structured questionnaire and Laboratory tests were performed on all the participants.

Consent and Ethical Approval

Ethical approval was obtained from the Health Research Ethic Committee (HREC) of ABUTH, with protocol number ABUTH/HREC/T09/2016. Written informed consent was obtained from the participants before the commencement of the study. Confidentiality of the participants was ensured. Participants that declined to participate were excluded from the study and without affectation of their standard of care.

Specimen Collection and Laboratory Analysis

Three millilitres (3ml) of blood sample was collected from each participant in K2 EDTA liquid BD vacutainer tube following standard aseptic procedures adopted from Dacie and Lewis[17] and incorporating the procedure as described by Becton.[18] Haemoglobin electrophoresis (using alkaline electrophoresis), HIV screening using Determine, complete blood counts (using Sysmex Haematology Analyser) and CD3+, CD4+ count (using Becton- Dickinson Immunocytometry Systems, FACS Count San Jose, CA, USA) were conducted on all samples within 6 hours of sample collection.

Data Processing/Analysis

Data generated were coded and soft copies kept in a password-protected computer. Data was cleaned and tested for normality. The quantitative data were expressed as mean ± SD or median. Frequency/percentages were used to determine socio-demographic variables. Chi-square was used to test for significance of the differences between distributions of socio-demographic variables of participants with SCA (HbSS) and apparently healthy control (HbAA) participants.

Independent sample t-test was used to test for differences in the variables (normally distributed) between participants with SCA (HbSS) and apparently healthy control (HbAA) while Mann-Whitney test was used to test for differences in the mean ranks of variables (not normally distributed) between patients with SCA (HbSS) and apparently healthy control (HbAA) participants.

Pearson's (parametric) and Spearman's rho (Nonparametric) correlations were used to establish relationship between Haematological indices with $CD3^+$ and $CD4^+$ T cells in blood samples of participants with SCA (HbSS) as the Study group and apparently healthy control (HbAA) participants as the Control group.

Statistical Package for Service and Solutions (SPSS Inc, Chicago IL) Software, version 23 (IBM Corporation for Windows) was used for the statistical

analysis**.** Level of significance was set at 95% confidence interval (CI) and p assumed to be ≤0.05.

RESULTS

Overall, a total of 60 participants were studied, out of which 30 participants had SCA (HbSS) i.e., "Study group," and 30 were apparently healthy blood donors (HbAA) i.e., "Controls" following alkaline electrophoresis.

Table 1: Haematological parameters of the Study group and Control

Parameter	Study Group n=30		Controls n=30		P Value [α]
	Median (IQR)	Mean(SD)	Median (IQR)	Mean(SD)	
Hb (g/dl)	8.40 (1.48)	-	12(2.50)	-	<0.0001
WBC (x 10^9/l)	-	12.30 ±3.5	-	6.51 ±2.1	<0.0001
Neutrophils	-	6.60 ±2.4	-	3.90 ±1.8	<0.0001
Plt (x 10^9/l)	434.00 (235.0)	-	354.5(164)	-	0.036

[α] Two tailed Independent T-test
Mann-Whitney U test;Plt=Platelets

All participants were seronegative for HIV-1 antibodies. There was no statistical difference between the Median age of the study group 22.5(7) years and 24.5 (9) years for the controls [p = 0.06]. Out of the study group 21 (70%) were females and 9 (30%) were males while there were 18 (60%) females and 12 (40%) males among the controls.

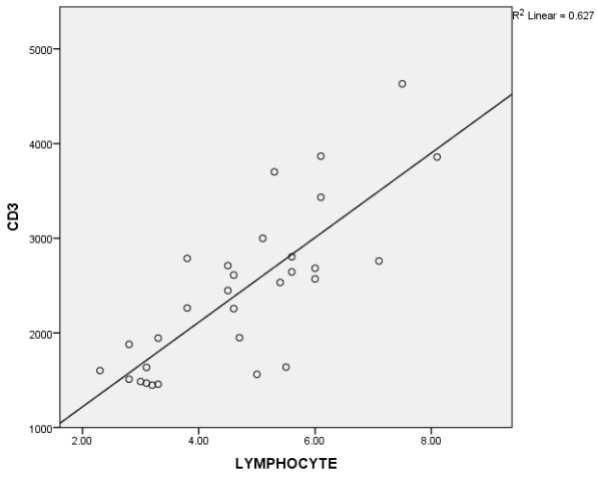

Figure 1: Correlation of ALC and CD3+ T-cell counts in Study group. Strong positive and significant correlation r= 0.79 p= < 0.0001

Table 2: Immunological Parameters of the Study group and Control

Parameter	Study Group n=30	Controls n=30	P Value [α]
	Mean ± (SD)	Mean ± (SD)	
ALC	4.73 ± 1.5	1.98 ±0.9	<0.0001
CD3$^+$(Cells/µL)	2438.13 ± 843.0	2672.93 ± 790.1	0.270*
CD4$^+$ (Cells/µL)	1363.83 ± 521.3	1696.87 ± 569.4	0.022

*Insignificant
[α] Two tailed Independent T-test

Table 3: Correlation between Haematological parameters and CD3$^+$ and CD4$^+$ T-cell subsets in study group

	Study Group, n=30			
	CD3$^+$		CD4$^+$	
	r	P-value	r	P-value
HGB	-0.140	0.461	**-0.309**	0.096[α]
WBC	**0.389**	0.034	0.221	0.241
NEUT	0.042	0.824	-0.083	0.663
PLT	-0.132	0.486	-0.059	0.759
ALC	**0.792**	<0.0001	**0.641**	<0.0001

r = Correlation coefficient
[α]Spearman's rho correlation (Non parametric)

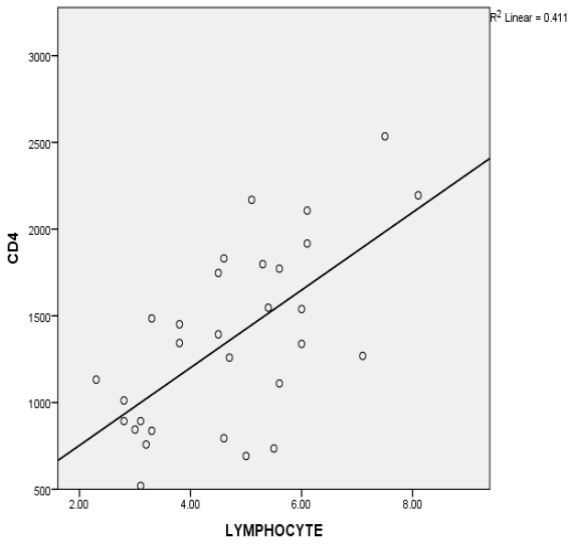

Figure 2: Correlation of ALC and CD4$^+$ T-Cell Counts in Study group. Strong Positive and significant correlation r 0.641 [p value < 0.0001]

Table 4: Correlation between Haematological parameters CD3+ and CD4+ T-cell subsets in Controls

	. Control, n=30			
	CD3+		CD4+	
	r	P-value	r	P-value
HGB	-0.169	0.372	-0.099	0.602
WBC	-0.01	0.957	-0.089	0.641
NEUT	0.023	0.906	-0.108	0.569
PLT	0.17	0.368	0.258	0.168α
ALC	-0.003	0.989	0.023	0.905

r = Correlation coefficient
αSpearman's rho correlation

DISCUSSION

In this study there were more females with SCA than males and this is consistent with the reports of Weiranga *et al*., (2001)[19] and Omoti, (2005)[20]; in their studies reported that, although SCA is inherited, it occurs more frequently in women than men. As this was a hospital-based study, this may be due to the higher incidence in the female gender on health care-seeking behaviour than men.[21]

The median age of the study group 22.5(7) years was similar to the findings of Akinbami *et al*., (2012)[22] and Omoti, (2005)[20] who reported the mean age of adults with SCD as 23.79 and 23.69 years respectively. However, this figure is lower than the findings of Anglin *et al*., (2009)[23] who reported the mean age of adult patients with SCD in the USA to be 39.50 years. Thus, indicating that SCA affects young adults who constitute the essence of school and workforce in northern Nigeria.

The lower haemoglobin concentration of the Study group which was significantly lower than that of the Controls is similar to the findings of Akinbami *et al*., (2012)[22] and Salawu *et al*., (2009)[24] and this has been associated with chronic haemolysis and higher susceptibility to infections. The findings in this study however differ [being lower] from what was observed by Omoti in Benin city during the comparison of 200 patients with SCD in steady state and 46 patients with SCD in vaso-occlusive crisis and 84 normal controls.[20] This difference in haemoglobin might have been due to 60% of the study group being transfused 3 to 4 months prior to the day of sample collection.[20]Expectedly the total white cell count was significantly higher in the Study group buttressing earlier findings by Akinbami *et al*., (2012)[22], Salawu *et al*., (2009)[24] and Musa *et al*., (2010)[15]. This has been explained to be as a result of the chronic underlying inflammation in patients with SCD, leading to the redistribution of leucocytes between the marginal and circulating pools of leucocytes.[22, 24] Although Fleming and de Silva (1996)[25] stated that people of African and Caribbean descent normally have lower Neutrophil count than people of other races due to a higher ratio of merging to circulating neutrophils, however the granulocyte count in this study was significantly higher in the Study group than the Controls. This also is consistent with the studies of Akinbami *et al*., (2012)[22], Salawu *et al*., (2009)[24] and Musa *et al*., (2010)[15]. Neutrophilic leucocytosis is more predominantly seen in SCD (Akinbami *et al*., 2012)[22], and it has been postulated that elevated Neutrophil count in SCD occurs as a result of redistribution of leucocytes as a result of stress in these patients. Elevated leukocyte counts are a marker of disease severity and have been correlated with poor outcome in patients with SCA.[26]In this study the significantly higher platelet count in patients with SCA compared to the apparently healthy controls, is contrary to the findings of Salawu *et al*., 2009[24] who reported lower but statistically non-significant mean platelet counts in asymptomatic patients with SCD. Musa *et al*., in 2010[15] showed no significant elevation of platelet counts in patients with SCD in the steady state. Minor episodes of microvascular occlusion occurring in the so called asymptomatic steady state may be insufficient to cause the overt painful crisis but can consume some platelets.[27]

In this study the significantly higher absolute lymphocytes count of the Study group is similar to the reports of Musa *et al*., (2010)[15] but in contrast to the findings by Adedeji, (1985)[28] in a study of 14 patients with SCA where he reported that the mean absolute lymphocyte count observed in patients with SCA were similar to that of Controls. However, this difference may not be unrelated to the larger sample size in our study.

Interestingly, despite the significantly high peripheral absolute lymphocyte count in the Study group, the CD3+ and CD4+ T cell subsets were reduced compared to the Controls, but this was only significant for the CD4+ T cells subsets (p = 0.022). Similar findings were reported by Adedeji (1985)[28] in a study of lymphocytes subpopulation in 14 patients with homozygous SCA. Musa *et al*., (2010)[15] also reported lower CD4+ T cells subsets between the patients with SCA and Controls. On the contrary, Ojo *et al*., (2014)[16] reported no significant difference in the number of CD4+ T lymphocyte counts between individuals with sickle cell anaemia and HbA (1016 ± 513 cells/μL vs. 920 ± 364cells/μL respectively). Although Koffi et al., (2003)[29] reported a reduced levels of T-cell subsets CD4+ and a significantly increased CD3+ cells (p=0.04) in patients with SCA, however, there was no significant difference in levels of CD4+ T cells (p= 0.05) between patients with SCA and the Control. The T-cell subpopulation and splenic status such as SCA-anemia induced splenic defects (autosplenectomy and splenomegaly) may be responsible.[29] Despite the increased ALC and reduced CD3+ and CD4+ T lymphocytes levels in this study, ALC shows a good and significant correlation with both CD3+ and CD4+

T lymphocytes. ALC has been correlated with CD4+T cells in several studies in patients with HIV infection, however the correlations were higher. [30, 31, 32] This study did not demonstrate correlations between ALC and other haematological parameters. Lymphocyte level is an index of cell-mediated immunity which is important in host defence against infections, malignancies and other autoimmune diseases.[11] Peripheral lymphocyte counts have been correlated with clinical stages and survival results in patients showing its prognostic values.[11] Therefore, this study as a baseline highlights the importance of relative and absolute numbers of T-lymphocyte subsets in patients with SCA.

In addition to the significantly low levels $CD4^+$ T lymphocytes subsets observed in this study, it is recommended that the functionality of $CD4^+$ T lymphocytes as well as the splenic status should be considered in further attempt to elucidate the cellular immune dysfunction in patients with SCA.

Declaration of conflict of interest

The authors declare no conflicts of interest with respect to the research, authorship, and / or publication of this article.

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